

**AMENDMENTS TO THE CLAIMS**

The following listing of the claims replaces all prior claim listings.

**Listing of Claims:**

Claim 1. (Withdrawn-Currently Amended) A process for cleaving a polypeptide comprising the steps of:

(1) providing a polypeptide comprising arginine or lysine at the P1 position of a desired cleavage site, an amino acid other than aspartic acid, glutamic acid or proline at the P1' position, and a single basic amino acid[[.]] or two or three consecutive basic amino acids situated at any site in the amino acid sequence from the P10 position to the P3 position or from the P3' position to the P5' position, (with the proviso that a single basic amino acid is not situated at the P6 or P4) wherein, if there is only a single basic amino acid situated in the amino acid sequence from the P10 position to the P3 position, the single basic amino acid is situated at a position other than the P6 or the P4 position; and

(2) cleaving the polypeptide with *E. coli* OmpT protease.

Claim 2. (Withdrawn-Currently Amended) [[A]] The process according to claim 1, wherein the polypeptide is a fusion protein comprising a protecting peptide and a target peptide wherein the C-terminus of the protecting peptide is the P1 position and the N-terminus of the protecting peptide is the P1' position , and wherein the fusion protein is produced by expressing a gene encoding the fusion protein in host cells in the step (1), and the fusion protein is cleaved with *E. coli* OmpT protease so as to liberate the target peptide in the step (2).

Claim 3. (Withdrawn-Currently Amended) The method process of claim 1 wherein, if a site which is not desired to be cleaved by *E. coli* OmpT protease is present in the polypeptide or the fusion protein, cleavage at said site is inhibited by situating an acidic amino acid at the P3 position of said site.

Claim 4. (Withdrawn-Currently Amended) The method process of claim 1, which comprises situating wherein two or three consecutive basic amino acids are situated between the P10 and P3 positions of the desired cleavage site in the polypeptide or fusion protein.

Claim 5. (Withdrawn-Currently Amended) The method process of claim 4, , which comprises situating wherein three consecutive basic amino acids are situated between the P5 and P3 positions of the desired cleavage site in the polypeptide or fusion protein.

Claim 6. (Withdrawn-Currently Amended) The method process of claim 1, wherein the basic amino acids are arginine and/or lysine.

Claim 7. (Withdrawn-Currently Amended) The method process of claim 6, wherein the basic amino acids are arginine.

Claim 8. (Withdrawn-Currently Amended) A polypeptide cleavage method wherein OmpT protease is used for cleavage at a desired cleavage site in the polypeptide, or a method for producing a target peptide which comprises cleavage at a desired cleavage site in a fusion protein, the method being characterized in that; A process for cleaving a polypeptide comprising cleaving a polypeptide at a desired cleavage site with *E. coli* OmpT protease, or producing a target peptide that comprises cleavage at a desired cleavage site in a fusion protein, wherein, if a site which is not desired to be cleaved by *E. coli* OmpT protease is present in said polypeptide or said fusion protein, cleavage at said site is inhibited by situating an acidic amino acid at the P3 position of said site.

Claim 9. (Withdrawn-Currently Amended) The method process of claim 8, wherein the acidic amino acid is aspartic acid.

Claim 10. (Withdrawn-Currently Amended) The method process of claim 1, wherein the amino acid sequence from the P5 to P1 positions of the desired cleavage site in the polypeptide or fusion protein is Arg-Arg-Arg-Ala-Arg (SEQ ID NO: 11).

Claim 11. (Withdrawn-Currently Amended) The method process of claim 1, wherein the amino acid sequence from the P7 to P1 positions of the desired cleavage site in the polypeptide or fusion protein is Asp-Ala-Arg-Arg-Arg-Ala-Arg (SEQ ID NO: 12).

Claim 12. (Currently Amended) A polypeptide cleavage method characterized by A process for cleaving a polypeptide comprising cleaving a desired cleavage site of a polypeptide or a fusion protein using an *E. coli* OmpT protease 97th amino acid variant to produce a target peptide, wherein the 97th amino acid from the N-terminus of the OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine.

Claim 13. (Withdrawn-Currently Amended). A polypeptide cleavage method characterized in that, when The process of claim 12, wherein the P1 position of the desired cleavage site in the polypeptide is arginine or lysine and the P1' position is an amino acid other than arginine or lysine, the desired cleavage site of said polypeptide is cleaved using an OmpT protease 97th amino acid variant wherein the 97th amino acid from the N terminus of the OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine.

Claim 14. (Withdrawn-Currently Amended) A polypeptide cleavage method characterized in that The process of claim 13, the P1 position of the desired cleavage site in the polypeptide is arginine or lysine, the P1' position is an amino acid other than arginine or lysine, wherein a single basic amino acid or two or three consecutive basic amino acids are situated at any site in the amino acid sequence from the P10 position to the P3 position or from the P3' position to the P5' position, and the desired cleavage site of said polypeptide is cleaved using an OmpT protease 97th amino acid variant wherein the 97th amino acid from the N terminus of the OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine.

Claim 15. (Currently Amended) A method for producing a target peptide, characterized by comprising transforming host cells with an expression plasmid having a gene coding for a fusion protein comprising a target peptide fused with a protecting peptide via a desired cleavage site that can be cleaved by an *E. coli* OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of the *E. coli* OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine, expressing said gene in said cells, and obtaining the target peptide from the fusion protein by cleavage with said protease at said cleavage site.

Claim 16. (Currently Amended) A method for producing a target peptide, characterized by comprising transforming host cells with an expression plasmid having a gene coding for a fusion protein comprising a protecting peptide whose C-terminus is arginine or lysine fused with a target peptide whose N-terminus is an amino acid other than arginine or lysine, via a desired cleavage site that can be cleaved by an *E. coli* OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of the *E. coli* OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine, expressing said gene in said cells, and obtaining the target peptide from the fusion protein by cleavage with said protease at said cleavage site.

Claim 17. (Withdrawn-Currently Amended) A method for producing a target peptide, characterized by The method of claim 16, transforming host cells with an expression plasmid having a gene coding for a fusion protein wherein a single basic amino acid or two or three consecutive basic amino acids are situated at any site in the amino acid sequence from the P10 position to the P3 position or from the P3' position to the P5' position at a desired cleavage site of a fusion protein comprising a protecting peptide whose C-terminus is arginine or lysine fused with a target peptide whose N-terminus is an amino acid other than arginine or lysine, via the cleavage site, and said desired cleavage site is a cleavage site that can be cleaved by an OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of the OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine,

glutamine, glutamic acid or histidine, expressing said gene in said cells, and obtaining the target peptide from the fusion protein by cleavage with said protease at said cleavage site.

Claim 18. (Withdrawn-Currently Amended) The method process of claim 12 wherein, if a site which is not desired to be cleaved by the *E. coli* OmpT protease 97th amino acid variant is present in the polypeptide or the fusion protein, cleavage at said site is inhibited by situating an acidic amino acid at the P3 position of said site.

Claim 19. (Withdrawn-Currently Amended) The method process of claim 12, which comprises situating wherein two or three consecutive basic amino acids are situated between the P10 and P3 positions of the desired cleavage site in the polypeptide or the fusion protein.

Claim 20. (Withdrawn-Currently Amended) The method process of claim 19, which comprises situating wherein three consecutive basic amino acids are situated between the P5 and P3 positions of the desired cleavage site in the polypeptide or the fusion protein.

Claim 21. (Withdrawn-Currently Amended) The method process of claim 14, wherein the basic amino acids are arginine and/or lysine.

Claim 22. (Withdrawn-Currently Amended) The method process of claim 21, wherein the basic amino acids are arginine.

Claim 23. (Currently Amended) A polypeptide cleavage method wherein an OmpT protease 97th amino acid variant is used for cleavage at a desired cleavage site in the polypeptide, or a method for producing a target peptide which comprises cleavage at a desired cleavage site in a fusion protein, the method being characterized in that, A process for cleaving a polypeptide comprising cleaving a polypeptide at a desired cleavage site with an *E. coli* OmpT protease 97th amino acid variant, or producing a target peptide that comprises cleavage at a desired cleavage site in a fusion protein, wherein if a site which is not desired to be cleaved by the *E. coli* OmpT protease 97th amino acid variant is present in said polypeptide or said fusion

protein, cleavage at said site is inhibited by situating an acidic amino acid at the P3 position of said site.

Claim 24. (Withdrawn-Currently Amended) The method process of claim 18, wherein the acidic amino acid is aspartic acid.

Claim 25. (Withdrawn-Currently Amended) The method process of claim 12, wherein the amino acid sequence from the P5 to P1 positions of the desired cleavage site in the polypeptide or the fusion protein is Arg-Arg-Arg-Ala-Arg (SEQ ID NO: 11).

Claim 26. (Currently Amended) The method process of claim 12, wherein the amino acid sequence from the P7 to P1 positions of the desired cleavage site in the polypeptide or the fusion protein is Asp-Ala-Arg-Arg-Arg-Ala-Arg (SEQ ID NO: 12).

Claim 27. (Currently Amended) The method process of claim 12, wherein the 97th amino acid from the N-terminus of the *E. coli* OmpT protease is leucine, methionine or histidine.

Claim 28. (Withdrawn-Currently Amended) The method process of claim 12, wherein the P1' position of the desired cleavage site of the polypeptide or the fusion protein or the N-terminus of the target peptide is serine or alanine, and the 97th amino acid of the *E. coli* OmpT protease 97th amino acid variant used is leucine.

Claim 29. (Withdrawn-Currently Amended) The method process of claim 12, wherein the P1' position of the desired cleavage site of the polypeptide or the fusion protein or the N-terminus of the target peptide is phenylalanine, alanine, serine, cysteine or tyrosine, and the 97th amino acid of the *E. coli* OmpT protease 97th amino acid variant used is methionine.

Claim 30. (Withdrawn-Currently Amended) The method process of claim 12, wherein the P1' position of the desired cleavage site of the polypeptide or the fusion protein or the N-terminus of the target peptide is alanine, valine, isoleucine, methionine, serine, threonine,

cysteine or asparagine, and the 97th amino acid of the *E. coli* OmpT protease 97th amino acid variant used is histidine.

Claim 31. (Withdrawn-Currently Amended) The method process of claim 2, wherein the target peptide is a peptide composed of between 22 and 45 amino acid residues.

Claim 32. (Withdrawn-Currently Amended) The method process of claim 31, wherein the target peptide is adrenocorticotropin hormone (1-24), motilin or calcitonin precursor.

Claim 33. (Withdrawn-Currently Amended) The method process of claim 2, wherein the host cells are *E. coli*.

Claim 34. (Withdrawn-Currently Amended) The method process of either claim 1 or claim 12, which comprises wherein the polypeptide is cleaved by using, as the cleaving protease, bacterial cells expressing a gene coding for *E. coli* OmpT protease or an *E. coli* OmpT protease 97th amino acid variant, and wherein the 97th amino acid from the N-terminus of *E. coli* OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine.

Claim 35. (Withdrawn-Currently Amended) The method process of either claim 1 or claim 12, which comprises co-expressing wherein a gene coding for *E. coli* OmpT protease or an *E. coli* OmpT protease 97th amino acid variant wherein the 97th amino acid from the N-terminus of *E. coli* OmpT protease is alanine, leucine, phenylalanine, methionine, serine, threonine, cysteine, asparagine, glutamine, glutamic acid or histidine, is co-expressed with and a gene coding for a polypeptide or fusion protein whose cleavage by said protease is desired.